



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification³ : A61K 31/15, 31/24, 31/155 A61K 31/165, 31/275; C07C123/00 C07C121/52, 119/20, 103/26	A1	(11) International Publication Number: WO 84/ 04676 (43) International Publication Date: 6 December 1984 (06.12.84)
(21) International Application Number: PCT/US84/00755 (22) International Filing Date: 21 May 1984 (21.05.84) (31) Priority Application Number: 497,370 (32) Priority Date: 23 May 1983 (23.05.83) (33) Priority Country: US (71)(72) Applicants and Inventors: VAN'T RIET, Bartholomeus [US/US]; 3419 Noble Avenue, Richmond, VA 23222 (US). WAMPLER, Galen, L. [US/US]; 6938 Chamberlayne Road, Mechanicsville, VA 23111 (US). ELFORD, Howard, L. [US/US]; 3313 Gloucester Road, Richmond, VA 23227 (US). (74) Agent: SCHELLIN, Eric, P.; Suite 301, 2001 Jefferson Davis Highway, Arlington, VA 22202 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), SU. Published <i>With international search report.</i>
(54) Title: POLYHYDROXYBENZOIC ACID DERIVATIVES (57) Abstract Polyhydroxybenzo-, polyhydroxyphenylaceto-, and polyhydroxymandelo-substituted amidines, amidates, amidoximes, and hydroxyamidoximes, also polyhydroxybenzonitriles and polyhydroxybenzamides; their syntheses, pharmaceutical formulation and uses as ribonucleotide reductase inhibitors, as free radical scavengers, and as mammalian antineoplastic agents.		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

-1-

Title

POLYHYDROXYBENZOIC ACID DERIVATIVES

Background of the Invention

5 Various hydroxy substituted benzohydroxamic acids are known--see Gale and Hynes, J. Med. Chem., 11, 191 (1968), Gale, Hynes and Smith, ibid, 13, 571 (1970), Van't Riet et al., United States Patent 4,263,322, Howle and Gale, Proc. Soc. Exptl. Biol. Med., 131, 697
10 (1969), Gale et al. Biochem. Pharm., 20, 2677 (1971).

Biological effects of hydroxy-substituted benzamides as well as other uses of such compounds are to found in Kreuchunas, United States Patent 2,849,480, Chemical Abstracts, 85, 94115w (1978), 74, 112752f
15 (1971).

The hydroxybenzohydroxamic acids are known to be inhibitors of ribonucleotide reductase according to the Elford, Wampler and Van't Riet group--see Cancer Res., 22, 589 (1979), Abstracts, Proc. Am. Assoc. Cancer Res., 18, 177 (1977), 19, 63 (1978), 20, 149 (1979),
20 22, 18 (1981), 23, 202 (1982), Va. J. Sci., 29, 81 (1978), papers, J. Pharm. Sci., 69, 856 (1980), New Approaches to the Design of Antineoplastic Agents- Bardos and Kalman, eds. pp 227 (Elsevier Biomedical, New York, N.Y.), 856 (1982), Advances in Enzyme Regulation, 19, 151 (1981), Abstract, Med. Ped. Oncol., 10
25 102 (1982), Abstract, Proc. 13th Int. Cancer Cong. pp 88 (1982), papers, J. Med. Chem., 22, 589 (1979) and Cancer Treat. Rep., 66, 1825 (1982).

30



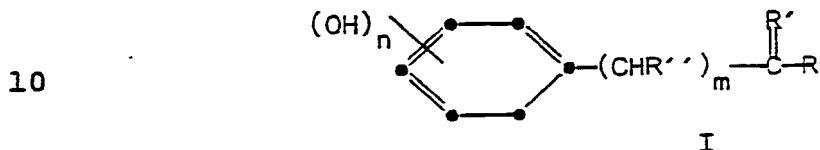
-2-

Polyhydroxy-substituted benzamidoximes, benzamidines, benzohydroxyamidoximes and benzamidates, so far as can be ascertained, are not recorded in the chemical literature.

5

Summary of the Invention

This invention provides compounds of the formula:



wherein n is 2-5, m is 0 or 1, R' is NOH or NH, R is NH₂ or NHOH when R' is NOH, R is NH₂ or O-C₁₋₃ alkyl when R' is NH, and R'' is H or OH; and pharmaceutically acceptable acid addition salts thereof.

Preferred compounds according to formula I are those in which at least two of the hydroxyls in the phenyl ring are vicinal. An even more preferred group of compounds are those in which the vicinal hydroxyls are at C-3 and C-4 of the phenyl ring.

Compounds according to I are named as hydroxy-substituted benzamidoximes when m is 0, R' is NOH and R is NH₂, as hydroxy-substituted benzohydroxyamidoximes when m is 0, R' is NOH and R is NHOH, as hydroxy-substituted benzamidates when m is 0, R' is NH and R is O-C₁₋₃ alkyl and as hydroxy-substituted benzamidines when m is 0, R' is NH and R is NH₂.

30



-3-

When m is 1, and R'' is OH, the compounds are named as hydroxy-substituted mandelamidoximes, mandelo-hydroxyamidoximes, mandelamidates and mandelamidines respectively. These mandelic acid derivatives have a center of assymetry and hence are prepared as a race-
5 mate or dl mixture. This invention includes both the racemates as well as the individual component optical isomers.

When m is 1 and R'' is H, the compounds are
10 named as hydroxy-substituted phenylacetamidoximes, phenylacetohydroxyamidoximes, phenylacetamidates and phenylacetamidines respectively.

Representative compounds according to formula I above include:

- 15 2,3-dihydroxybenzohydroxyamidoxime
- 3,4-dihydroxybenzohydroxyamidoxime
- methyl 2,3,4-trihydroxybenzamidate
- isopropyl 3,5-dihydroxybenzamidate
- ethyl 3,4,5-trihydroxybenzamidate
- 20 ethyl 3,4-dihydroxybenzamidate
- 3,4-dihydroxybenzamidoxime
- 3,4,5-trihydroxybenzamidoxime
- 2,3,5-trihydroxybenzamidoxime
- n-propyl 2,4,5-trihydroxybenzamidate
- 25 2,3-dihydroxybenzamidoxime
- ethyl 2,4-dihydroxybenzamidate
- ethyl 3,4,5-trihydroxybenzamidate
- 2,5-dihydroxybenzohydroxyamidoxime
- 3,4,5-trihydroxybenzamidine
- 30 2,3-dihydroxybenzamidine



-4-

2,3,4-trihydroxybenzamidine
3,4-dihydroxybenzamidine
dl-2,4,5-trihydroxymandelamidine
2,4,5-trihydroxybenzamidine
5 3,5-dihydroxybenzamidine
dl-3,4-dihydroxymandelamidine
dl-3,4,5-trihydroxymandelamidine
dl-2,3-dihydroxymandelamidine
dl-2,3,4-trihydroxymandelohydroxyamidoxime
10 dl-2,4,5-trihydroxymandelohydroxyamidoxime
2,3,4-trihydroxybenzamidoxime
2,3,4-trihydroxybenzohydroxyamidoxime
3,4,5-trihydroxybenzohydroxyamidoxime
ethyl 2,3-dihydroxybenzamidate
15 dl-3,4-dihydroxymandelamidoxime
dl-3,4,5-trihydroxymandelohydroxyamidoxime
dl-3,5-dihydroxymandelohydroxyamidoxime
dl-3,4-dihydroxymandelohydroxyamidoxime
dl-2,4-dihydroxymandelamidoxime
20 dl-2,3-dihydroxymandelamidoxime
dl-3,5-dihydroxymandelamidoxime
dl-2,3,4-trihydroxymandelamidoxime
dl-3,4,5-trihydroxymandelamidoxime
dl-2,4,5-trihydroxymandelamidoxime
25 dl-methyl 3,4-dihydroxymandelamidate
dl-ethyl 3,5-dihydroxymandelamidate
dl-n-propyl 2,3-dihydroxymandelamidate
dl-methyl 2,3,4-trihydroxymandelamidate
dl-ethyl 3,4,5-trihydroxymandelamidate
30 dl-ethyl 2,3,5-trihydroxymandelamidate
dl-isopropyl 2,4,5-trihydroxymandelamidate



-5-

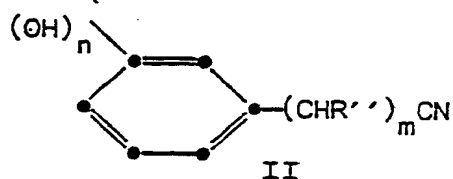
3,4-dihydroxyphenylacetamidoxime
3,5-dihydroxyphenylacetamidoxime
2,3-dihydroxyphenylacetamidoxime
2,5-dihydroxyphenylacetamidoxime
5 2,4-dihydroxyphenylacetamidoxime
2,3,4-trihydroxyphenylacetamidoxime
3,4,5-trihydroxyphenylacetamidoxime
2,4,5-trihydroxyphenylacetamidoxime
2,3,5-trihydroxyphenylacetamidoxime
10 ethyl 3,4-dihydroxyphenylacetamidate
methyl 2,3-dihydroxyphenylacetamidate
n-propyl 3,5-dihydroxyphenylacetamidate
isopropyl 2,3,4-trihydroxyphenylacetamidate
methyl 3,4,5-trihydroxyphenylacetamidate
15 methyl 2,4,5-trihydroxyphenylacetamidate
methyl 2,3,4,5-tetrahydroxyphenylacetamidate
ethyl 2,3,4,6-tetrahydroxyphenylacetamidate
methyl 2,3,4,5,6-pentahydroxyphenylacetamidate
2,3,4,5-tetrahydroxyphenylacetamidine
20 2,3,4,6-tetrahydroxybenzylacetamidine
2,3,5,6-tetrahydroxyphenylacetamidine
2,3,4,5,6-pentahydroxyphenylacetamidine
2,3,4,5,6-pentahydroxyphenylacetohydroxyamidoxime
2,3,5,6-tetrahydroxyphenylacetohydroxyamidoxime
2,3,4,5-tetrahydroxyphenylacetohydroxyamidoxime
25 ethyl 2,3,5-trihydroxyphenylacetamidate
2,3-dihydroxyphenylacetamidine
3,4-dihydroxyphenylacetamidine
3,5-dihydroxyphenylacetamidine
2,4-dihydroxyphenylacetamidine
30 2,5-dihydroxyphenylacetamidine



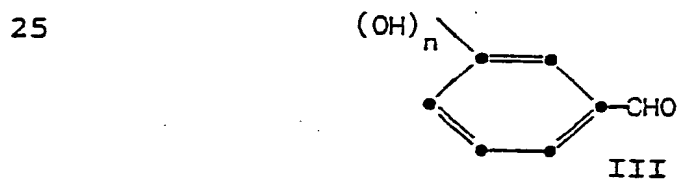
-6-

2,3,4-trihydroxyphenylacetamidine
 3,4,5-trihydroxyphenylacetamidine
 2,4,5-trihydroxyphenylacetamidine
 2,3,5-trihydroxyphenylacetamidine
 5 3,4-dihydroxyphenylacetohydroxyamidoxime
 3,5-dihydroxyphenylacetohydroxyamidoxime
 2,3-dihydroxyphenylacetohydroxyamidoxime
 2,3,4-trihydroxyphenylacetohydroxyamidoxime
 2,3,5-trihydroxyphenylacetohydroxyamidoxime
 10 2,4,5-trihydroxyphenylacetohydroxyamidoxime
 3,4,5-trihydroxyphenylacetohydroxyamidoxime,
 and the like.

Benzamidoximes and phenylacetamidoximes
 according to I above, where R' is NOH and R is NH₂,
 15 can be prepared reacting a nitrile of the formula



20 where m is 0 or 1 and R' is H with hydroxylamine in
 aqueous solution. Where R' is OH and m is 1, this
 nitrile may be a transitory intermediate formed by
 reacting an aldehyde of the formula



25 where n is 2-5 with a mixture of an alkali metal
 30 cyanide and hydroxylamine hydrochloride in aqueous



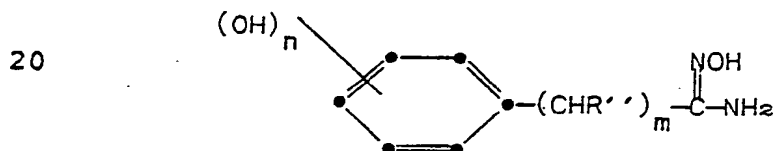
-7-

solution. The mandelonitrile (a cyanohydrin) forms initially but then reacts at once with hydroxylamine to give the desired mandelamidoxime.

The amidates (I where R is O-C₁₋₃ alkyl and R' is NH) can be prepared by reacting the above nitrile (II) with a lower alkanol (C₁₋₃ alkylOH) to which has been added an acid such as gaseous HCl. The reaction medium here should be non-aqueous, and Lewis acids other than HCl can be employed.

Nitriles useful in the above two synthetic procedures are readily available by processes set forth in the art. 2,3,4-Trihydroxybenzonitrile, the three tetrahydroxybenzonitriles and pentahydroxybenzonitrile are, however, new and their synthesis will be set forth in detail below.

Compounds according to I in which R is NHOH and R' is NOH can be prepared from the corresponding benzamidoxime, phenylacetamidoxime or mandelamidoxime



when n, m and R'' have the same significance as before by reaction with hydroxylamine hydrochloride. The hydroxy-substituted benzamidines, phenylacetamidines and mandelamidines can be prepared from the corresponding amidate by reaction with ethanolic ammonia.

30



-8-

The following more detailed examples illustrate the preparation of the compounds of this invention. Other equally useful methods for their preparation will readily suggest themselves to those skilled in the art.

Example 1

Preparation of 3,4-Dihydroxybenzamidoxime

30 g. of 3,4-Dihydroxybenzonitrile were dissolved in 300 ml. of water containing 25 g. of hydroxylaminesulfate which had been neutralized by the addition of aqueous sodium hydroxide to pH = 8.0. The reaction mixture was stirred at about 45° C. for 18 hours. 3,4-Dihydroxybenzamidoxime formed in the above reaction had precipitated and was collected by filtration. The filtered amidoxime was suspended in water and the aqueous suspension acidified to about pH = 2.0 with 12N aqueous hydrochloric acid. The acidic solution was decolorized with charcoal and the solvent removed by evaporation. Recrystallization of the residue yielded 3,4-dihydroxybenzamidoximehydrochloride melting at about 193° C. with decomposition.

Analysis Calculated: C, 41.09; H, 4.43; N, 13.69

Found: C, 41.12; H, 4.47; N, 13.69

Equivalent weight by titration 206 (theory = 204.6);
yield = 72%

30



Example 2

Preparation of 3,4,5-Trihydroxybenzaminoxime

5 About 7.5 g. of 3,4,5-trihydroxybenzonitrile
were dissolved in 200 ml. of water containing 7 g. of
hydroxylamine sulfate which solution had previously
been neutralized to about pH = 8.0 with aqueous sodium
hydroxide. 2 g. of sodium sulfite were also present in
10 solution. The reaction mixture was stirred at 45°C.
for about 18 hours after which time the precipitated
3,4,5-trihydroxybenzaminoxime formed in the above
reaction was collected. The product was converted to
the hydrochloride salt and the hydrochloride salt
purified by the process of Example 1. 3,4,5-Tri-
15 hydroxybenzaminoxime hydrochloride thus prepared melted
with decomposition at a temperature of about 206°C.
after recrystallization from an isopropanol-ethyl
acetate solvent mixture.

Analysis Calculated: C, 38.11; H, 4.11; N, 12.72

20 Found: C, 38.16; H, 4.16; N, 12.66

Equivalent weight by titration with aqueous sodium
hydroxide = 220 (theory = 220.6); yield = 80%

Example 3

25 Preparation of Ethyl 3,4,5-Trihydroxybenzamidate

5 g. of 3,4,5-Trihydroxybenzonitrile were
dissolved in ether. 2.2 ml. of ethanol were added.
Next, anhydrous gaseous hydrogen chloride was passed
through the solution. Ethyl 3,4,5-trihydroxybenzamidate
30 hydrochloride formed in the above reaction precipitated.



-10-

The precipitate was recrystallized from an isopropanol-ether solvent mixture. Ethyl 3,4,5-trihydroxybenzamidate hydrochloride thus prepared and purified melted at about 172°C. with decomposition.

5 Analysis Calculated: C, 46.26; H, 5.18; N, 5.99
 Found: C, 46.26; H, 5.22; N, 6.00

Equivalent weight by titration with sodium hydroxide = 115.5 (theory = 116.8); yield = 78%

10 Ethyl 3,4,5-trihydroxybenzamidate can be prepared by neutralization of the hydrochloride salt, extraction of the ester into ether and removal of the ether by evaporation.

Example 4

15 Preparation of Ethyl 3,4-Dihydroxybenzamidate

 Following the procedure of Example 3, 3,4-dihydroxybenzonitrile was converted to ethyl 3,4-dihydroxybenzamidate hydrochloride by the reaction with ethanol in the presence of hydrogen chloride. The
20 compound melted at 170° C. with decomposition after recrystallization from an isopropanol/ether solvent mixture; yield = 65%

 Analysis Calculated: C, 49.67; H, 5.56; N, 6.44
25 Found: C, 49.91; H, 5.61; N, 6.45

30

-11-

Example 5

Preparation of Gallamidine

5 About 4.5 g. of ethyl 3,4,5-trihydroxybenz-
amidate hydrochloride were heated with an excess of 14N
aqueous ammonium hydroxide in ethanol solution. The
volatile constituents were removed by evaporation and
the residue, comprising gallamidine formed in the above
reaction, was dissolved in alcohol. Gallamidine free
10 base was converted to the hydrochloride salt by passing
gaseous hydrogen chloride into the alcoholic solution.
Gallamidine hydrochloride melted at about 169°C. with
decomposition after recrystallization from an ethanol/
ethyl acetate solvent mixture; yield = 53%

15 Equivalent weight = 203 by titration with aqueous
sodium hydroxide (theory 204.5)

Analysis Calculated: C, 41.09; H, 4.43; N, 13.69;

Found: C, 40.80; H, 4.47; N, 14.30.

Example 6

20 Preparation of 3,4-Dihydroxybenzohydroxyamidoxime

A solution of 5.5 g. of 3,4-dihydroxy-
benzamidoxime (from Example 1) was prepared in a
minimal quantity of methanol. 3.5 g. of hydroxylamine
hydrochloride were added. The reaction mixture was
25 allowed to stand for about one day at about 50°C.
Volatile constituents were removed by evaporation.
Ethyl acetate was added to the residue. The resulting
precipitate was separated by filtration and gaseous
hydrogen chloride passed into the filtrate. 3,4-

-12-

Dihydroxybenzohydroxyamidoxime hydrochloride thus prepared was separated by filtration. The compound melted at about 169°C. with decomposition.

5 Equivalent weight by titration with sodium hydroxide = 219 (theory = 220.5)

Analysis Calculated: C, 38.11; H, 4.11; N, 12.70;
Found: C, 38.28; H, 4.15; N, 12.61.

Example 7

10 Preparation of 3,4-Dihydroxymandelamidoxime

Seven and eight-tenths grams of 3,4-dihydroxybenzaldehyde were added to 100 ml. of an aqueous solution held at -15°C. containing 7.8 g. of hydroxylamine hydrochloride and 5.5 g. of sodium cyanide. The reaction
15 mixture was stirred overnight at about 0°C. and then filtered. Four g. of 3,4-dihydroxymandelamidoxime monohydrate formed in the above reaction were obtained melting at 151°C. with decomposition (after loss of
20 water of crystallization at about 120°C) yield = 36%

Analysis Calculated: C, 44.44; H, 5.60; N, 12.96;
Found: C, 44.37; H, 5.65; N, 12.92.

Example 8

25 Preparation of 2,3,4-Trihydroxybenzamidoxime

Following the procedure of Example 2, 3.5 g. of 2,3,4-trihydroxybenzonitrile were reacted in 60 ml. of water with 4 g. of hydroxylamine sulfate and 2 g. of sodium bisulfite at pH = 8.0 (adjusted by addition
30 of concentrated aqueous sodium hydroxide). The re-



-13-

action mixture was maintained at ambient temperature for about one hour and was then cooled. 2,3,4-Trihydroxybenzamidoxime precipitated and the precipitate was collected by filtration. The filter cake was dissolved in dilute aqueous hydrochloric acid and the resulting solution filtered through activated charcoal. Evaporation of the volatile constituents from the filtrate under reduced pressure yielded 2,3,4-trihydroxybenzamidoxime as a hydrochloride salt, melting at 207°C. with decomposition after recrystallization from a methanol/ethyl acetate solvent mixture; yield = 9%.

Analysis Calculated: C, 38.11; H, 4.11; N, 12.72
Found: C, 37.34; H, 4.06; N, 12.43.

The preparation of nitrile starting materials useful for preparing most of the compounds of this invention where the amide starting material is available or known is illustrated in the following example.

Preparation I

Preparation of Nitrile Starting Materials

A reaction mixture containing 23.5 g. of gallamide, 180 ml. of ethyl acetate and 35 ml. of thionylchloride was refluxed for about 18 hours. The volatile contents were removed by evaporation in vacuo and the resulting residue dissolved in 215 ml. of water. This aqueous solution was heated to about 90°C. until the evolution of gas had ceased. The aqueous solution was filtered through charcoal and the water



-14-

removed from the filtrate by evaporation. 18 g. of Gallonitrile were obtained melting at about 219°C. with decomposition (86% yield). Other nitriles useful as starting materials can be prepared similarly.

5

Preparation II

Preparation of 2,3,4-Trihydroxybenzonitrile

Eighteen and five tenths grams of 2,3,4-trihydroxybenzamide were refluxed with 20 ml. of phosphorus oxychloride and 100 ml. of ethyl acetate for 2.5 hours. The volatile constituents were removed in vacuo and the residue poured into 150 ml. of an ice-water mixture. The resulting suspension was heated to 95°C. and then filtered through activated carbon. The filtrate was concentrated to about 50 ml. whereupon 2,3,4-trihydroxybenzonitrile precipitated and was collected by filtration. 2,3,4-Trihydroxybenzonitrile monohydrate thus prepared melted at 172°C. after recrystallization from a methanol-benzene solvent mixture; yield = 57%

Analysis Calculated: C, 49.71; H, 4.17; N, 8.28

Found: C, 49.70; H, 4.17; N, 8.27.

Preparation III

25 Preparation of 2,3,4,5-Tetrahydroxybenzonitrile

Following the procedure of Mayer et al., Chem. Ber., 89, 511 (1956), 3,4,5-trimethoxybenzoic acid was brominated in ethyl acetate solution without added water using only 40% of the chloroform volume specified in that reference. Twenty-three and five



-15-

tenths grams of 2-bromo-3,4,5-trimethoxybenzoic acid thus synthesized were added to a solution prepared by dissolving 1 g. of cupric acetate and 5 g. of sodium sulfite in 110 ml. of water followed by 27 g. of sodium hydroxide. The reaction mixture, which was a suspension, was heated to boiling for about six hours. Since it showed a tendency to solidify, extra water was added to maintain fluidity. At the end of six hours, the suspension was poured into a mixture of ice and 120 ml. of 12N aqueous hydrochloric acid. The volume was raised to about 900 ml. with water. This mixture was then heated. Complete dissolution occurred at about 95°C at which point, 700 mg. of thioacetamide were added and heating continued until black copper sulfide had formed. The mixture was filtered through carbon. 3,4,5-Trimethoxysalicylic acid monohydrate precipitated from the filtrate.

The corresponding methyl ester was prepared by heating 3,4,5-trimethoxysalicylic acid monohydrate in methanol containing 2% sulfuric acid, as set forth in the above reference.

Methyl 3,4,5-trimethoxysalicylate prepared as above was heated in 14N aqueous ammonium hydroxide for four hours. The volatile constituents were removed in vacuo and the resulting residue, 3,4,5-trimethoxysalicylamide, was recrystallized from water at pH = 5.0. The compound melted at about 151°C.; yield = 70% (13.4 g. of 3,4,5-trimethoxy salicylic acid gave 9.3 g. of pure 3,4,5-trimethoxysalicylamide.) Seven and seven tenths grams of 3,4,5-trimethoxysalicylamide were



-16-

heated to reflux with 8 ml. of phosphorusoxychloride in 100 ml. of ethyl acetate for about one hour. The volatile constituents were removed by evaporation in vacuo and the residue, comprising 3,4,5-trimethoxy-salicylnitrile, was dissolved in water to which some ethanol was added until the solution was clear at 95°C. Again the volatile constituents were removed in vacuo and the liquid residue dissolved in hot benzene. The residue was dried and the solution used in the next demethylation step without further purification.

The above benzene solution was diluted to 100 ml. with benzene and 20 g. of anhydrous aluminum chloride added. The reaction mixture was heated to reflux temperature for two hours and the suspension poured into a mixture of 12N hydrochloric acid and ice. The volatile constituents were removed from this mixture by evaporation and 2,3,4,5-tetrahydroxybenzonitrile, formed in the above reaction, was extracted into ethyl acetate. The ethyl acetate extract was dried and the residue obtained by removal of the ethyl acetate was recrystallized from water. Three grams of 2,3,4,5-tetrahydroxybenzonitrile were obtained which decomposed at 219°C. yield = 36% from the starting 3,4,5-trimethoxysalicylamide.

25 Analysis Calculated (for one-fourth mole of water):

C, 48.99; H, 3.02; N, 8.16

Found: C, 49.06; H, 3.23; N, 8.14.

30



-17-

2,3,4,5-tetrahydroxybenzamide was prepared by careful hydrolysis of the corresponding nitrile in concentrated hydrochloric acid at 60°C. The amide gradually precipitated from a solution of 1.5 g. of the nitrile in 25 ml. of hydrochloric acid. After recrystallization from water, the product decomposed at 290°C. Yield = 23%.

Analysis Calculated: C, 45.41; H, 3.81; N, 7.56

Found: C, 44.83; H, 3.88; N, 7.27.

The above procedure can be adapted for the preparation of other tetrahydroxybenzonitriles, although isomer separation procedures may be necessary where two monobromo substitution products are possible in the starting trimethoxybenzoic acid. Thus, 2,3,5,6-tetrahydroxybenzonitrile can be prepared from 2,3,5-trimethoxybenzoic acid (with 2,3,4,5-tetrahydroxybenzonitrile as a contaminant). Likewise, 2,3,4,6-tetrahydroxybenzonitrile can be prepared from 2,3,4-trimethoxybenzoic acid.

Preparation IV

Preparation of Pentahydroxybenzonitrile

Eleven and three-tenths grams of penta-methoxybenzamide obtained by the procedure of Dallacher, Ann., 665, 78-83 (1963) were dissolved in 50 ml. of ethyl acetate to which 10 ml. of thionyl chloride had been added. The reaction mixture was heated to reflux for about three hours. Evaporation of volatile constituents in vacuo yielded a residue comprising penta-



-18-

methoxybenzonitrile melting at 64°C. after recrystallization from methanol/water; yield = 80%

Analysis Calculated: C, 56.92; H, 5.97; N, 5.53
Found: C, 56.92; H, 5.88; N, 5.49.

5 Four and three-tenths grams of pentamethoxybenzonitrile were refluxed in 12.5 g. of anhydrous aluminum chloride and 125 ml. of toluene for three hours after which time the suspension was poured into a mixture of 50 ml. of 12N aqueous hydrochloric acid and
10 200 g. of ice. The toluene layer was separated and the product recovered from the aqueous layer by filtration. Recrystallization from an ethyl acetate/toluene solvent mixture yielded pentahydroxybenzonitrile decomposing at 238°C. with a prior slight decomposition at 220°C.;
15 yield = 66%

Analysis Calculated (for monohydrate):

C, 41.80; H, 3.51; N, 6.96
Found: C, 42.20; H, 3.54; N, 6.94.

20 Following the procedure of Example 3, ethyl tetrahydroxybenzamidate can be prepared from the corresponding nitrile.

Also following the above procedure, pentahydroxybenzamidate can be prepared from the corresponding pentahydroxybenzonitrile by reaction with
25 ethanol in the presence of anhydrous HCl. The compound is converted to the hydrochloride salt and isolated as such according to the procedure of Example 3.

30



-19-

Compounds represented by formula I above have the ability to inhibit ribonucleotide reductase, an enzyme involved in the reductive conversion of ribonucleotides to deoxyribonucleotides. This enzymatic reaction is a rate controlling step in the biosynthetic pathway leading to DNA and cell replication. In general, the ribonucleotide reductase level is closely correlated with cellular replication. Thus, it is not surprising that the compounds of this invention, which are potent ribonucleotide reductase inhibitors, are also capable of prolonging the life of mice carrying transplanted tumors since replication of tumor cells is equally inhibited. In particular, we have found that administration of a compound of this invention coming within the scope of formula I above prolongs the life of mice inoculated with Ll210 leukemia, a tumor not ordinarily susceptible to chemotherapy. In addition, the compounds have shown activity against P388 leukemia and B16 melanoma.

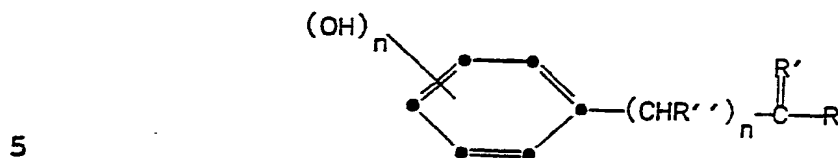
The results of biological tests of compounds according to formula I are incorporated in a series of Tables which follow. Table 1 gives ribonucleotide reductase data for representative compounds of formula I. In the table, column 1 gives the substitution pattern in the benzene ring, column 2, the (CHR'')_m group, column 3, the R' group, and column 4, the C-R

ID₅₀ (inhibitory dose in micromolar concentration which inhibits ribonucleotide reductase by 50%) in μ moles.



-20-

Table 1



	(OH) _n	(CHR'') _m	R' "C-R	ID ₅₀ μM
10	3,4	bond	NOH "C-NH ₂ ·HCl	8
	3,4	bond	NH "C-OEt·HCl	12
15	3,4	bond	NH "C-NH ₂ ·HCl	20
	3,4	bond	NOH "C-NHOH·HCl	40
20	3,4,5	bond	NOH "C-NH ₂ ·HCl	5
	3,4,5	bond	NH "C-OEt·HCl	15
25	3,4,5	bond	NH "C-NH ₂ ·HCl	25
	3,4	CHOH	NOH "C-NH ₂ ·H ₂ O	10
30	3,4,5	bond	NOH "C-NHOH·HCl	25
	2,3,4	bond	NOH "C-NH ₂ ·HCl	7
	2,3	bond	NOH "C-NH ₂ ·HCl	18



-21-

In the above determination of ID_{50} 's in Table 1, ribonucleotide reductase is partially purified from HeLa cells or Ehrlich ascites cells by a procedure similar to that set forth by Elford et al. J. Biol. Chem., 245, 5228 (1970). The activity of the enzyme was measured by a slightly modified assay procedure originally developed by Reichard et al. id., 236, 1150 (1969). This procedure measures the conversion of CDP to dCDP. The assay mixture (0.34 ml.) contains 3 μ Ci of [3H] CDP (specific activity 14-19 Ci/ μ mol), 3.3 μ mole ATP, 5.9 μ moles magnesium chloride, 8.8 μ moles Hepes buffer at pH = 7.5, 15 μ moles dithiothreitol and enzyme protein between 0.4 and 1.3 mg. Incubation was provided for forty minutes at 30°C. Ion exchange chromatography employing Dowex 50 (H^+) resin is used to separate the product from the substrate. The inhibitors were dissolved in water and a mixture of water and up to 1% ethanol or 2% dimethylsulfoxide, neither one of which inhibited the enzyme at these concentrations. Each inhibitor was tested at a minimum of three concentrations and the active compounds reassayed at least one additional time. ID_{50} 's (μ molar) were estimated from graphs summarizing results for each compound.

Testing of the compounds of this invention against L-1210 lymphoid leukemia were carried out as follows: L-1210 leukemia was maintained by weekly passage of 10^5 L-1210 cells intraperitoneally into DBA/2 mice. Diluted ascitic fluid, 0.1 ml. (10^5 cells), was administered ip to female B6D₂F₁ mice weighing about 20 g. Drugs were administered ip 24



-22-

hours after tumor transplantation and injections were continued daily for a total of eight days. A group of control mice receiving only the injection medium were maintained. Table 2 which follows gives the antitumor activity against L-1210 leukemia for certain compounds of this invention. In the table, column 1 gives the name of the compound, column 2 the dose in mg./kg., and column 3 the percent increase in survival time over controls at each dose level.

10

15

20

25

30



-23-

Table 2
ANTITUMOR ACTIVITY IN L1210 LEUKEMIA

Name of Compound	Dose in mg/kg	% Increase in Survival Time
3,4-dihydroxybenzamidoxime - HCl	157	132.0
	200	43.5
	238	46.9
	300	73.9
3,4,5-dihydroxybenzamidoxime - HCl	59	90.0
	132	54.5
	196	toxic
	220	63.6
	275	30.6
2,3,4-trihydroxybenzamidoxime - HCl	100	38.9
	200	36.1
	218	67.7
	300	toxic
ethyl 3,4-dihydroxybenzamidate - HCl	218	toxic
	327	toxic
	435	toxic
ethyl 3,4,5-trihydroxybenzamidate - HCl	300	42.3
	450	58.4
3,4-dihydroxybenzamidine - HCl	52	26.8
	100	58.3
	104	34.1
	200	41.7
	300	47.2
	400	47.8
603	83.0	



-24-

Table 2 continued

<u>Name of Compound</u>	<u>Dose in mg/kg</u>	<u>% Increase in Survival Time</u>
3,4,5-trihydroxybenzamidine - HCl	200	43.5
	300	10.2
3,4-dihydroxybenzohydroxyamidoxime - HCl	155	39.0
	200	47.8
	300	41.3
3,4,5-trihydroxybenzohydroxyamidoxime - HCl	100	47.2
	200	55.6
	300	63.9
3,4-dihydroxybenzohydroxymandel amidoxime - H ₂ O	435	45.2
	606	45.2
2,3-dihydroxybenzamidoxime - HCl	300	toxic



-25-

3,4-Dihydroxybenzamidoxime and 3,4,5-tri-hydroxybenzamidoxime were subjected to a further series of tests against various transplanted tumors in mice according to the following protocols:

5 For L-1210 lymphoid leukemia, 20 grams CDF₁ or BDF₁ mice rejected ip with 10⁵ leukemia cells. The drug was given one day after tumor inoculation continued for an additional eight days. The mean survival time for the treated animal was compared to that of the control group. Table 3 which follows gives the results of this test for the above two compounds. In the table, column 1 gives the name of the compound, column 2 the dose levels, and column 3 treated over control percent survival time. (In other words, a figure of 15 175 indicates a 75% increase in survival time.)

20

25

30



-26-

5
10
15
20
25
30

Table 3

Activity vs L-1210 Leukemia

<u>Name of Compound</u>	<u>Dose mg/kg</u>	<u>t/c%</u>
3,4-dihydroxybenzamidoxime·HCl	400	toxic, 200*202
	300	113
	200	175, 128, 155, 186
	100	128, 132, 141, 133
	50	137, 117, 132, 146
	25	130
3,4,5-trihydroxybenzamidoxime·HCl	200	212, 182
	100	152, 137
	50	138, 137
	25	136, 121

*t/c% greater than 130 are significant



-27-

The drugs were also used to treat melanotic melanoma B₁₆. The protocol for testing against this tumor is as follows:

0.5 ml. of a tumor homogenate prepared by
5 homogenizing 1 g. of tumor with 10 ml. of cold balanced
solution is implanted ip in groups of 10 B₆C₃F₁ mice.
The drug is administered daily for a total of nine days
starting one day after tumor inoculation. The results
are expressed as mean survival time of treated group
10 versus control group (T/C) as a percent. Table 4 which
follows gives the results of this test.

15

20

25

30



-28-

5

10

15

20

25

30

Table 4

Activity vs B₁₆ Melanoma

<u>Name of Compound</u>	<u>Dose mg/kg</u>	<u>T/C%</u>
3,4-dihydroxybenzamidoxime·HCl	400	117
	200	134
	100	129
	50	127
	25	119
ethyl gallamidate	800	toxic
	400	124, 156, 137
	200	121, 125, 122
	100	121, 109, 107
	50	109, 104
	25	105

* T/C% greater than 120 are significant.



-29-

A compound of this invention was also tested on the solid colon tumor model, colon 38. The tumor was implanted subcutaneously and the drug injected intraperitoneally twice a day on day two (seven hours
5 apart) and twice on day nine. The median tumor weight estimated from tumor diameter is the parameter of tumor inhibition and is measured on day 20. The median tumor weight of treated versus control = T/C percent. Table 5 which follows gives the results of this test.

10

15

20

25

30



-30-

5

10

15

20

25

30

Table 5

Activity vs Colon 38

<u>Name of Compound</u>	<u>Dose mg/kg</u>	<u>T/C%</u>
3,4-dihydroxybenzamidoxime·HCl	800	87

Lower doses were ineffective.



-31-

Compounds of this invention also showed some activity against P388 leukemia. The following protocol was used:

CDF₁ mice were injected ip with 10⁶ cells.

5 The drug was administered in the first day after tumor inoculation and continued daily for five treatments. The mean survival time of the treated group compared to the non-treated control group equals $T/C \times 100 = T/C$ percent. Table 6 which follows gives the results of
10 these determinations.

15

20

25

30



-32-

5

10

15

20

25

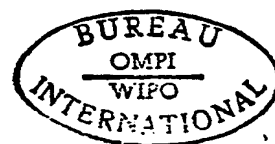
30

Table 6

Activity vs P388 Leukemia

<u>Name of Compound</u>	<u>Dose mg/kg</u>	<u>T/C*</u>
3,4,5-trihydroxybenzamidoxime·HCl	400	toxic
	300	163
	200	155, 155, 177
	100	150, 154, 138, 148
	50	142, 138, 141
ethyl gallamidate	400	149, 160
	200	139, 123
	100	129, 128
	50	114
	25	112
	12.5	104

*T/C's over 130 are considered significant



-33-

It is believed that the antineoplastic activity of the compounds of this invention is due in part to their ability to scavenge free-radicals. Additionally, the compounds of this invention are cancer protective agents and this utility also may be a manifestation of their free-radical scavenging capability. Free-radical scavenging agents may also be useful in detoxifying mammals in whom an excess of free radicals is a cause and/or result of the toxicity. Other possible uses of free radical scavengers are to inhibit prostaglandin transformation, leucotriene interconversion and lipogenesis, to act as inflammatory modulators and as food additive or preservatives to prevent lipid oxidation. The free radical scavenging ability of the compounds of the invention was determined by measuring the destruction of the stable free radical, diphenylpicrylhydrazyl, in the presence of the test compound in a manner similar to that reported by Venker and Herzmann, Naturwiss, 47, 133-134 (1960). The absorbance at 518 nm of a 100 μ M solution of diphenylpicrylhydrazyl free radical in acetone was monitored in a Gilford spectrophotometer. The test compound was added at a final concentration of 25 μ M and the rate of reduction of absorption at 518 nm was observed. Table 7 below gives the free radical scavenging abilities of the compounds of this invention expressed as the initial rate of decrease in optical density units/min.

30



-34-

Table 7
FREE RADICAL SCAVENGING ABILITIES

<u>Compound</u>		Initial Δ units/min.* (518 nm)
5	3,4-dihydroxymandelamidoxime	0.383
	3,4-dihydroxybenzamidoxime·HCl	0.718
	3,4,5-trihydroxybenzamidoxime·HCl	0.929
	Ethyl 3,4,5-trihydroxybenzamidate·HCl	3.730
10	Ethyl 3,4-dihydroxybenzamidate·HCl	0.706
	3,4-dihydroxybenzamidine·HCl	1.721
	3,4,5-trihydroxybenzohydroxyamidoxime·HCl	0.882
	3,4,5-trihydroxybenzamidine·HCl	4.912

*One optical density unit decrease of initial
15 absorbance/min at 518 nm by 2.5 μ M of agent.

20

25

30



-35-

Additional free radical scavenging potential of a compound of this invention was measured on a generated tyrosine free radical. There is evidence that a tyrosine free radical is formed as part of the mammalian ribonucleotide reductase enzyme during the conversion of ribonucleotides to deoxyribonucleotides--see L. Åkerblom, et al. Proc. Natl. Acad. Sci. USA, 78, 2159-2163 (1981). The method for the generation of the tyrosine free radical and its destruction by a compound of this invention was accomplished by pulse radiolysis experiments at Brunel University, Oxbridge England on the Brunel 4 MeV linear accelerator using a 1.5 cm optical cell and a 200 ns pulse delivering a dose of approximately 1 krad as measured by thiocyanate dosimetry. All solutions were purged with nitrogen using a syringe-deaerating technique prior to use. The detailed description of the methodology can be found in R.L. Willson, Chemistry and Industry, 183-193 (1977).

For the generation of the tyrosine radical, sodium azide was utilized as an intermediate between the pulse radiolysis generated free electron and tyrosine. Hydroxyurea was used as a reference point for the ability of these compounds to scavenge tyrosine free radical since hydroxyurea inhibition of ribonucleotide reductase has been attributed to its ability to scavenge the free radical of the active mammalian ribonucleotide reductase.--See Åkerblom et al (loc. cit). See also I.K. Larsen et al., Eur. J. Biochem, 125, 75-81 (1982). A rate constant of $1.9 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ was determined for hydroxyurea and $4.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$



-36-

S⁻¹ for 3,4-dihydroxybenzamidoxime hydrochloride. In other words, 3,4-dihydroxybenzamidoxime hydrochloride was 100+ times a faster scavenger of tyrosine free radical than hydroxyurea.

5 The novel nitriles and amide starting materials for the synthesis of the compounds of this invention, (Formula I wherein n is 4 or 5), although chiefly useful as intermediates, have ribonucleotide reductase activity and are also active free radical
10 scavengers.

Table 8 which follows gives anti-tumor data
vs L-1210 and P-388 leukemia for these novel nitriles.

15

20

25

30



-37-

Table 8
Antileukemia Activity

L1210

5	<u>Name of Compound</u>	<u>Dose in mg/kg</u>	<u>To Increase in Survival Time</u>
	3,4-dihydroxybenzonitrile	47.6	4.7
		95.2	15.6
		196	Toxic
	2,3,4-trihydroxy- benzonitrile	50	11.0
10		100	26.0
		155	12.1
		259	Toxic
		327	Toxic
	3,4,5-trihydroxy- benzonitrile	118	34.0
		157	28.0
15	2,3,4,5-tetrahydroxy- benzonitrile	163	48.4
		230	Toxic
	pentahydroxybenzonitrile	34.3	14.2
		51.5	0
		77.2	Toxic
20		116.0	Toxic

P-388

	3,4,5-trihydroxy- benzonitrile	400	126
		200	126, 139
25		100	110, 139
		50	115, 120

30



-38-

The compounds of this invention are administered parenterally to mammals suffering from a neoplastic disease, preferably using a water soluble salt of the drug. Intravenous injection of an isotonic salt solution of the drug salt is the preferred route of administration.

10

15

20

25

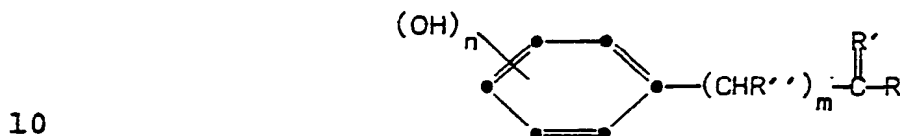
30



-39-

We claim:

1. A method of inhibiting ribonucleotide reductase which comprises administering to a mammal carrying a tumor having a relatively high ribonucleo-
 5 tide reductase level, an amount of a compound according to the following formula effective to inhibit ribonucleotide reductase



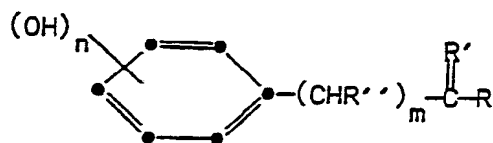
wherein n is 2-5, m is 0 or 1, R' is NOH or NH, R is NH₂ or NHOH when R' is NOH; R is NH₂ or O-C₁₋₃ alkyl when R' is NH; and R'' is H or OH or a pharmaceutically acceptable acid addition salt thereof.

2. A method according to claim 1 in which 3,4-dihydroxybenzamidoxime is administered.
3. A method according to claim 1 in which 3,4,5-trihydroxybenzamidoxime is administered.
- 20 4. A method according to claim 1 in which ethyl gallamidate is administered.
5. A method according to claim 1 in which two of the OH groups in the phenyl ring are vicinal.
6. A method according to claim 1 in which n
 25 is 2 or 3 and R is NHOH.
7. A method according to claim 1 in which m is 0.
8. A method according to claim 1 in which R is NHOH.
- 30 9. A method according to claim 1 in which 3,4-dihydroxybenzamidine is administered.



-40-

10. A compound of the formula



in which n is 2-5, m is 0 or 1, R' is NH or NOH, R is NH₂ or NHOH; when R' is NOH; R is NH₂ or O-C₁₋₃ alkyl; when R' is NH; R'' is H or OH, or a pharmaceutically acceptable acid addition salt thereof.

10 11. A compound according to claim 10, said compound being 3,4-dihydroxybenzamidoxime hydrochloride.

12. A compound according to claim 10, said compound being 3,4,5-trihydroxybenzamidoxime hydrochloride.

15 13. A compound according to claim 10, said compound being ethyl gallamate.

14. A compound according to claim 10, said compound being 2,3,4-trihydroxybenzamidoxime.

15. A compound according to claim 10, said compound being 3,4,5-trihydroxybenzamidoxime.

20 16. A compound according to claim 10, said compound being 3,4-dihydroxybenzamidine.

17. A compound according to claim 10, said compound being 3,4-dihydroxybenzohydroxyamidoxime.

25 18. A compound according to claim 10, said compound being 3,4,5-trihydroxybenzohydroxyamidoxime.

19. A compound according to claim 10 in which n is 3 and the three hydroxyls are in the 2, 3 and 4 positions.

30 20. A compound according to claim 10 in which n is 2 and the hydroxyls are in the 2 and 3 positions.



-41-

21. A hydrochloride salt of a compound according to claim 10.

22. A compound according to claim 10 in which two of the hydroxyls are vicinal.

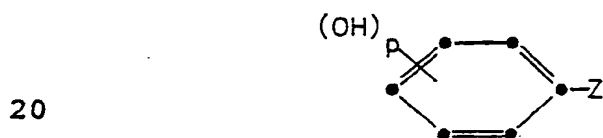
5 23. A compound according to claim 10 in which n is 2 and the two hydroxyls are in the 3 and 4 positions.

24. A compound according to claim 10 in which n is 3 and the three hydroxyls are in the 3, 4 and 5 positions.

10 25. A compound according to claim 10 in which n is 2 or 3, two hydroxyls are vicinal and m is 0.

26. The method of scavenging free radicals in vivo which comprises administering to a mammal having a disease condition involving excess free radical formation, and in need of treatment, an effective dose of a
15 compound according to Claim 10.

27. A compound of the formula



wherein p is 4 or 5 wherein Z is CN or CONH₂.

28. A compound according to claim 27, said
25 compound being 2,3,4,5-tetrahydroxybenzonitrile.

29. A compound according to claim 27, said compound being pentahydroxybenzonitrile.

30. A compound according to claim 27, said compound being 2,3,4,5-tetrahydroxybenzamide.

30.



-42-

31. The method of treating leukemia in mammals which comprises administering to a mammal suffering from leukemia and in need of treatment, an anti-leukemic dose of a compound according to claim 10.

5 32. The method according to claim 31 in which 3,4-dihydroxybenzamidoxime is administered.

33. A method according to claim 31 in which 3,4,5-trihydroxybenzamidoxime is administered.

10 34. A method according to Claim 31 in which 3,4-dihydroxybenzamidine is administered.

35. A pharmaceutical formulation in unit dosage form for parenteral administration containing a compound according to claim 10 plus one or more pharmaceutically-acceptable excipients.

15 36. The method of treating leukemia in mammals which comprises administering to a mammal suffering from leukemia and in need of treatment, an anti-leukemia dose of a compound according to claim 27.

20

25

30



INTERNATIONAL SEARCH REPORT

International Application No PCT/US84/00755

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC ³ A61K-31/15; 31/24; 31/155; 31/165 and 31/275 C07C-123/00; 121/52; 119/20; and 103/26		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	260/453.7 and 465F 564/170; 229; and 247 424/298; 304; 324; 326; and 327	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
Chemical Abstracts and CAS Online		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	N, Advances in Enzyme Regulation, Volume 19, published 1981 by Pergamon (New York, NY, U.S.A.) Elford, Howard L. et al., 'Regulation of Ribonucleotide Reductase in Mammalian Cells by Chemotherapeutic Agents.' Pages 151-168. Page 152 of particular relevancy.	1-3, 5-8, 10-12, 14, 15, 17-26, 31-33 & 35
X	N, Cancer Research, Volume 39, published 1979 by International Cancer Research Foundation (Philadelphia, PA, U.S.A.), Elford, Howard L. et al., 'New Ribonucleotide Reductase Inhibitors with Antineoplastic Activity', Pages 844-851. Page 850 of particular interest.	27, 30 & 36
X	US, A, 3,629,443, published December 1971 Lafon et al., See formula Column 1	17, 18 & 35
X	US, A, 4,263,322, published April 21, 1981 van't Riet et al., Column 4 of particular relevancy.	27, 30 & 36
A	GB, A, 358,279, published October 8, 1931, Carpmael	27, 29 & 30
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹		Date of Mailing of this International Search Report ²
8 August 1984		09 AUG 1984
International Searching Authority ¹		Signature of Authorized Officer ¹⁹
ISA/US		Leah Hendriksen <i>Leah Hendriksen</i>

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X N, Chemical Abstracts, Volume 97, No. 19
issued November 8, 1982 (Columbus, Ohio,
U.S.A.) Wagner, G. et al. 'Preparation
of phenol-protein-conjugates by reaction
of hydroxybenzimidic acid esters with
proteins' see page 522, column 2, the
abstract no. 160,748f. Pharmazie 1982,
37(4) 251-3 (Ger.)

10, 13 &
19-25

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

Six (6) inventions were set forth in the Office action
mailed July 2, 1984.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)